Medical and Pediatric Oncology Supplement 1:25–28 (1996)

Genetics of Retinoblastoma: Implications for Other Human Cancers

David W. Yandell, ScD, and Christopher Poremba, MD

INTRODUCTION

The childhood tumor retinoblastoma, and the associated cancer predisposition syndrome that underlies many cases of this disease, have become a paradigm for the role that tumor suppressor genes play in human cancers and in inherited cancer predisposition syndromes. Although retinoblastoma is rare in comparison to most adult cancers, with an incidence of 1/20,000 births or lower, it is the most common childhood eye cancer and the eighth most common tumor in children younger than 15 [1,2]. This potentially fatal tumor almost always strikes children younger than 6 years old. The disease can be treated successfully in the majority of cases. Steady improvements over the last 30 years in both surgical and nonsurgical interventions have lowered mortality and have improved the quality of life for most survivors.

Our success in clinical management of retinoblastoma has been paralleled by remarkable strides forward in our understanding of the genetic aspects of the disease. It is now possible to use DNA testing to distinguish the hereditary from nonhereditary forms in some cases where clinical evaluation cannot differentiate the two forms, and to carry out presymptomatic DNA-based carrier testing for many families [3]. Even more important, a careful look at both the underlying genetic features of the disease and their clinical and practical implications can tell us a great deal about what to expect in dealing with other, more common cancer predisposition syndromes.

THE GENETICS OF RETINOBLASTOMA

The genetics of retinoblastoma are complex. The tumor occurs in both hereditary and nonhereditary forms, with approximately 40% of all cases being associated with a dominantly inherited predisposition to the disease. Two-thirds of those children who are hereditary carriers will develop one or more independent primary retinal tumors, often involving both eyes. Carriers of the hereditary form appear to be at significantly elevated risk later in life for developing other spontaneous or treatment-related second tumors, usually of mesenchymal origin [1,4]. In particular, they have a high risk for soft-tissue and bone tumors, a risk that triples if their retinoblastoma is treated with ionizing radiation. Patients with the nonhereditary form of retinoblastoma develop only one primary eye tumor, though intraocular seeding may occur. The two forms can often be differentiated by either the presence of bilateral eye tumors at first presentation (or metachronous involvement of the second eye) or a previous family history of retinoblastoma (approximately 10% of all cases of retinoblastoma occur in children with an affected relative), either of which is diagnostic for hereditary disease. However, three out of four children with the hereditary form have a negative family history, and many of them will have only one tumor at first presentation [2].

To make matters even more complicated, the disease is incompletely penetrant in 10% of the persons who inherit a defective gene. While these nonpenetrant hereditary carriers do not develop eye tumors, they are no less likely than affected carriers to transmit a predisposition to retinoblastoma to their progeny. These genetic complexities, including a high new germinal mutation rate, incomplete penetrance, and clinically indistinguishable but genetically distinct forms of the disease in many patients complicate decisions for the family and the clinician alike related to treatment, prognosis, surveillance, and family planning.

In addition, recent evidence suggests that the likelihood of transmission of the inherited form of the disease may be slightly different when passed through males vs. females [5]. The mechanism underlying such non-Mendelian transmission is unknown. It is well established that the vast majority of new germline mutations causing this disease are of paternal origin but the basis for this is likewise unclear [6]. As compared to the genetic complexities of the disease described above, however, the clinical implications of any possible deviation from strict Mendelian transmission are virtually nil; likewise, the observed bias in the parental origin of new mutations has

From the Department of Pathology, University of Vermont College of Medicine, Burlington, Vermont (D.W.Y.); Department of Pathology, Gerhard-Domagk-Institut für Pathologie, University of Münster, Münster, Germany (C.P.)

Address reprint requests to David W. Yandell, Sc.D., A-179 Medical Alumni Bldg., Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05405.

minor implications in risk counseling but no major impact for most patients and their families.

MUTATIONS CAUSING RETINOBLASTOMA

As Knudson originally postulated, it is now known that the hereditary form of retinoblastoma occurs in persons who carry one defective copy of the retinoblastoma (RB) gene as a constitutional defect [7]. A "second hit" must occur to inactivate the remaining functional copy of the RB gene in a retinal cell before it will grow unchecked and become a tumor. Whereas all retinal target cells in a carrier of the hereditary form of the disease require only one "hit" to become tumorigenic, the same cell in a noncarrier (a person with two normal copies of the gene) requires two "hits" to reach the same degree of malignant potential. Thus, the high probability of multiple tumor formation in carriers vs. extremely low probability of even a single tumor in noncarriers appears to be driven stochastically—two "hits" in a single cell are less likely than one "hit."

Isolation and cloning of the RB gene in 1987 has, since then, enabled a detailed analysis of the mutations that lead to formation of this tumor. A variety of techniques have been used to characterize the spectrum of mutations occurring in retinoblastoma tumors. Most of the mutations that have been revealed are single-base changes (substitution, insertion, or deletion) or deletions less than 20 bp [8,9]. About 10–20% of RB gene mutations are larger in size (50 bases to 50,000 bases) and must be detected by conventional Southern blotting or by newer techniques utilizing the polymerase chain reaction. Only about 1–2% are large enough to be detected by conventional cytogenetic evaluation, which is limited to DNA deletions or rearrangements involving a few million base pairs or more.

In our laboratory, where we have characterized more than 200 mutations in retinoblastoma tumors and in blood specimens from children with the hereditary form of the cancer, we have found no evidence for hotspots for point mutation or structural rearrangement in the RB gene. Most inherited mutations are "private," occurring at different sites in unrelated individuals. The vast majority of all mutations identified to date appear to cause a total loss of function of the protein (so-called nonsense mutations). Only a small number of mutations described so far cause a changed protein due to an amino acid substitution (so-called missense mutations), and several of these appear to be quite special in the way they cause disease. In particular, substitution of an arginine with a tryptophan at codon 661 causes a form of hereditary retinoblastoma in which there is an unusually high proportion of unaffected or unilaterally affected carriers [10,11]. We and others have found this mutation in numerous families characterized by very low penetrance for the disease. It is not unusual in such families to find unaffected adult carriers of this mutation who, after careful funduscopic examination, show evidence of one or more regressed retinal lesions [12]. It is likely that these persons had undiagnosed benign or superficial retinal tumors in childhood which regressed or did not develop full malignant potential. Although several different "low penetrance" mutations have been reported, the rule appears to be that most persons with the hereditary form of retinoblastoma carry nonsense mutations in one copy of the gene, and will have a predictable probability of developing the disease.

DNA-BASED DIAGNOSIS AND CARRIER TESTING FOR RETINOBLASTOMA

Because of the complex genetics of this disease, DNA-based carrier assessment can be very helpful to the clinician in determining the likelihood that an affected child will develop additional tumors. DNA testing can also be used to determine whether healthy siblings of an affected child are at risk for the disease, the probability that a couple with one affected child will have for bearing a second child with the disease, and for prenatal testing in pregnancies involving either a parent who was affected as a child or unaffected parents who have one or more affected children [3,9,13]. Because most mutations causing the disease are unique to each affected child or family, DNA-testing cannot be used in the absence of at least one affected person in a family (i.e., it has no application in routine screening of the healthy population). It also has limited practical application in situations demanding immediate turnaround, such as for prenatal testing, unless a full DNA evaluation of the family has been conducted prior to the pregnancy. Because the cost of a full DNA workup can be several thousand dollars, with typical laboratory turnaround times of 3 to 6 months or more, practical concerns limit the usefulness of this technology for many families. Still, the initial workup need be done only once for a given family and does not need to be repeated; after this point, all subsequent carrier testing of siblings, relatives, or future pregnancies can be done expeditiously and at a minimal cost per test.

The lessons related to DNA testing that can be drawn from our experience so far with retinoblastoma are these: 1) DNA testing can have a significant impact on the way care is provided to families with a potentially high risk for cancer. It can facilitate better surveillance and management for those who will be affected, and reduce unnecessary surveillance in those who are not carriers. 2) DNA testing can permit informed decisions about family planning in potentially cancer-prone families. This includes the identification of unaffected adult carriers who may be at elevated risk for bearing an affected child, and the identification of healthy noncarriers who may suffer from (and often make reproductive decisions based on) the mistaken assumption that they are at risk of bearing children with the disease. 3) Although feasible in principle, the high cost and technical difficulty of DNA testing for genetic cancer predisposition still limits its widespread application regardless of the potential clinical value. 4)

Even if the technology were widely available at low cost, the interpretation and clinical implications of some DNA test results will be foreign to many clinicians, who will need guidance or training in order to make the best use of the information.

TUMOR SUPPRESSOR GENE INVOLVEMENT IN CANCER PREDISPOSITION

Tumor suppressor genes, as a class, can be loosely defined as genes that play a role in restricting growth or cell cycle progression in a differentiated cell type or tissue. Unlike the dominant oncogenes, in which specific mutations can lead to activation of a new and oncogenic form of the protein, tumor suppressor genes play a role in oncogenesis primarily due to inactivation or abrogation of their normal function. As described above for the retinoblastoma gene, "two hit" kinetics involving inactivation of one, then the other, copy of a tumor suppressor gene seems to be the rule with respect to their role in oncogenesis [14]. Because loss of one copy of the gene usually has no phenotypic consequences at the cellular level, the constitutional presence of one defective copy of the gene in all cells of a developing embryo has little or no consequences during embryogenesis (we now know that exceptions to this generalization exist, including some constitutional genetic defects associated with Wilms' tumor in children). For this reason, tumor suppressor genes may be the most common class of gene leading to excess inherited cancer risk. Recent discoveries bear this out, and a number of tumor suppressor loci associated with cancer predisposition syndromes have been cloned and characterized including genes associated with Wilms' tumor, the neurofibromatoses, familial adenomatous polyposis, Li-Fraumeni syndrome, familial breast and ovarian cancer, Von Hippel Lindau syndrome, and others.

The prototypical example of a tumor suppressor is the retinoblastoma gene, which plays a fundamental role in regulation of cell cycle progression and in apoptosis (the process of programmed cell death) [15]. Mutations of the retinoblastoma gene have been found in a variety of tumor types in addition to retinoblastoma. These include carcinomas of the bladder, breast, prostate, liver, lung, cervix, soft tissue and osteosarcomas, leukemias, and multiple myeloma [reviewed in 14]. There is no question that in many human tissues, normal RB function is a fundamental necessity for controlled growth while abrogation of its activity leads to loss of growth control. It is not unreasonable to think that several of the well-documented genetic characteristics of this disease summarized above, such as "two hit" inactivation kinetics, a preponderance of loss of function mutations, incomplete or variable disease penetrance, and the prevalence of many different family-specific "private" mutations among hereditary carriers (rather than a small number of common mutations) will also hold true for other cancer predisposition syndromes associated with inherited tumor suppressor gene mutations.

If this reasoning based on retinoblastoma is even partially correct, it has several practical implications. First, as in retinoblastoma, we should not assume that all persons who are cancer prone due to inherited genetic defects will present with a family history of disease. This makes sense, as the "reproductive fitness" (the relative likelihood of having offspring, as compared to the general population) of many persons who are cancer prone may be low, especially if the cancers normally arise in childhood. Large, multigeneration families affected by such syndromes may thus be the exception rather than the rule. In retinoblastoma, the ratio of new hereditary cases to those that have an established family history in previous generations is roughly 3:1. This ratio is bound to vary with the particulars of cancer predisposition associated with different tumor suppressor genes. Studies so far on p53-related cancer predisposition suggest that this prediction may be at least partially correct [16]. In the absence of a clear family history of cancer as a warning sign for hereditary disease, clinicians must be alert for alternative warning signs. Examples include a personal history of multiple histologically distinct primary cancers, treatment-related second cancers, an unusually early age of onset, and the occurrence of solid tumors in early childhood.

A second implication is that clinical integration of DNA testing for other more common cancer predisposition syndromes may be costly and difficult as has been the case with retinoblastoma. For the reasons cited above, it is likely there will be a preponderance of "private" mutations in many syndromes caused by tumor suppressor genes, with unrelated families carrying different defects. This makes testing difficult in each new patient or family, since the genetic target is large and no assumptions can be made about the location or type of alteration that is carried. These factors will make large-scale screening of unaffected persons infeasible. Studies so far of cancer-prone families with p53 gene defects, and with adenomatous polyposis coli, support this argument [16,17]. A notable exception to this pattern may be the gene associated with most cases of early onset familial breast and ovarian cancer, BRCA1, for which a relatively small number of mutations have been found. The early evidence suggests that perhaps a majority of affected women from certain ethnic groups may carry one of a small number of mutations. This will perhaps enable cost-effective screening of large numbers of women when inexpensive assays designed to detect these common mutations become available.

INCOMPLETE PENETRANCE OF CANCER PREDISPOSITION SYNDROMES

Perhaps the most important inference we can draw from the genetics of retinoblastoma is that penetrance of

the disease phenotype will be incomplete for many tumor suppressor gene-related cancer predisposition syndromes; disease penetrance may be determined by the type of mutation carried by a person. Evidence from familial adenomatous polyposis again supports this argument [17]. The age of onset and severity of this disease vary dramatically depending on the location and type of mutation in the APC tumor suppressor gene. This will complicate the interpretation of DNA diagnostic testing for such syndromes, and may have important implications for surveillance and intervention in affected families. However, as argued above for retinoblastoma, it is likely that many such syndromes will commonly be associated with inherited gene mutations that grossly alter or block function of the tumor suppressor gene in most cases. If correct, this inference carries with it the likelihood that we will not be able to predict easily what sort of disease predisposition will be conferred by more subtle alterations in the protein. Amino acid substitutions conferring only a small change in the protein might be genetically neutral polymorphisms, or they might confer a significantly elevated risk for the disease. By analogy to the low penetrance retinoblastoma families, however, such gene carriers might not be affected by more than a single tumor, and the disease would frequently skip generations. A disease as rare as retinoblastoma makes it easy to spot low penetrance transmission by a careful review of the family history. With more common cancers such as breast cancer or colon cancer, a disease penetrance of 10–20% would be exceedingly difficult to identify as familial cancer. For example, if subtle alterations in BRCA1 exist, and these are as weakly penetrant as the rare low penetrance retinoblastoma gene mutations, these mutation carriers will be hard to find. The excess risk for breast cancer these persons would carry and will transmit to half their offspring is not insignificant, however. Integration of large scale DNA screening programs for such genetic risk factors for common cancers would present logistical and ethical problems, yet the clinical value of identifying such high risk individuals could be great.

CONCLUSION

The wealth of knowledge gained about the genetics of retinoblastoma in the last few years provide important insights about the genetics of more common cancers. As new cancer-causing genes are cloned in the future, and as we begin to traverse the difficult terrain of premorbid genetic risk assessment for common diseases, it will serve us well to look back on the progress and problems associated with translation of these earlier research findings into useful clinical tests. Our technology for large scale genetic screening must improve. Information about a patient's intrinsic genetic risk for cancer is useful both to the clinician, in decisions related to treatment and surveillance, and to the patient for informed family planning. Our ability to interpret this information on a wider scale must also improve. This should involve the creation of opportunities for oncologists to receive training in the genetics of common cancers, and training in the use and interpretation of DNA diagnostic tests.

REFERENCES

1. Vogel F: Genetics of retinoblastoma. Hum Genet 52:1–54, 1979.
2. Silverberg E, Lubera J: Cancer statistics. CA 36:9–25, 1986.
3. Yandell DW, Campbell TA, Dayton SH, Petersen R, Walton D, Little JB, McKonkie-Rosell A, Buckley EG, Dryja TP: Identification of cancer-causing point mutations in the human retinoblastoma gene and application to genetic counseling. N Engl J Med 321:1689–1695, 1989.
4. Tamboli A, Podgor MJ, Horm JW: The incidence of retinoblastoma in the United States: 1974 through 1985. Arch Ophthalmol 108:128–132, 1990.
5. Munier FL, Arabien L, Flodman P, Spence MA: Putative non-Mendelian transmission of retinoblastoma in males: a phenotypic segregation analysis of 150 pedigrees. Hum Genet 94:484–490, 1994.
6. Dryja TP, Mukai S, Petersen R, Rapaport JM, Walton D, Yandell DW: Parental origin of mutations of the retinoblastoma gene. Nature 339:556–558, 1989.
7. Knudson AG: Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820–823, 1971.
8. Dunn JM, Phillips RA, Zhu X, Becker A, Gallie BL: Mutations in the RB1 gene and their effects on transcription. Mol Cell Biol 9:4596–4604, 1989.
9. Shimizu T, Toguchida T, Kato MV, Kaneko A, Ishizaki K, Sasaki MS: Detection of mutations of the RB1 gene in retinoblastoma patients by using exon-by-exon PCR-SSCP analysis. Am J Hum Genet 54:793–800, 1994.
10. Hogg A, Bia B, Onadim Z, Cowell JK: Molecular mechanisms of oncogenic mutations in tumors from patients with bilateral and unilateral retinoblastoma. Proc Natl Acad Sci USA 90:7351–7355, 1993.
11. Lohman DR, Brandt B, Hopping W, Passarge E, Horsthemke B: Distinct RB1 mutations with low penetrance in hereditary retinoblastoma. Hum Genet 94:349–354, 1994.
12. Onadim Z, Hykin PG, Hungerford JL, Cowell AH: Genetic counselling in retinoblastoma: importance of ocular fundus examination of first degree relatives and linkage analysis. Br J Ophthalmol 75:147–150, 1991.
13. Draper GJ, Sanders BM, Brownbill PA, Hawkins MM: Patterns of risk of hereditary retinoblastoma and applications to genetic counseling. Br J Cancer 66:211–219, 1992.
14. Schubert EL, Hansen MF, Strong LC: The retinoblastoma gene and its significance. Ann Med 16:177–184, 1994.
15. Ewen M: The cell cycle and the retinoblastoma protein family. Cancer Metastasis Rev 13:45–66, 1994.
16. Toguchida J, Yamaguchi T, Dayton S, Beauchump R, Herrerra G, Ishizaki K, Yamamuro T, Meyers PA, Little JB, Sasaki MS, Weichselbaum RR, Yandell DW: Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. N Engl J Med 326:1301–1308, 1992.
17. Lynch HT, Watson P, Smyrk TC, Lanspa SJ, Boman BM, Boland CR, Lynch JF, Cavalieri RJ, Leppert M, White R, Sidransky D, Vogelstein B: Colon cancer genetics. Cancer [suppl] 70:1300–1312, 1992.